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D) extending the oligonucleotide primer along the first template, by means of a polymerase having strand displacement activity, to form a second template complementary to the first template, thereby displacing the first region from the first complementary region and displacing the nucleic acid chain formed during said synthesizing from the first template; and

- E) displacing the second template from the first template.
- 30. The method according to claim 29, wherein the 5' terminal of the oligonucleotide primer in step C) comprises a nucleotide sequence complementary to the first region of the first template.
- 31. The method according to claim 29, wherein said second template has (i) a 3' end portion comprising the second region and the second complementary region which, under suitable conditions, anneal to one another to form the second loop, (ii) a 5' end portion comprising the first region and the first complementary region which, under suitable conditions, anneal to one another to form the first loop, and (iii) a single-stranded target complement region connecting the 3' end portion and the 5' end portion, said method further comprising:
- F) synthesizing a second nucleic acid chain complementary to the single-stranded target complement region of the second template using the 3' terminal of the second template, when the second region and second complementary region are annealed to one another, as the origin of said synthesizing;
- G) annealing to the second loop of the second template a second oligonucleotide primer comprising at the 3' terminal a nucleotide sequence complementary to the second loop of the second template; and
- H) extending the second oligonucleotide primer along the second template, by means of a polymerase having strand displacement activity, to form a third template which is substantially the same as the first template, thereby displacing the second region from the second complementary region on the second template and displacing the second nucleic acid chain formed during said synthesizing in step F) from the second template.
- 32. The method according to claim 31, wherein the 5' terminal of the second oligonucleotide primer in step G) comprises a nucleotide sequence complementary to the second region of the second template.
- 33. The method according to claim 31, wherein the second nucleic acid chain synthesized in step F) is in the same strand as the second template.







- 34. The method according to claim 31 further comprising:I) displacing the third template from the second template.
- 35. The method according to claim 34 further comprising: repeating steps B) through I) using the third template to form a fourth template which is substantially the same as the second template and a fifth template which is substantially the same as the first and third templates.
- 36. The method according to claim 29, wherein the nucleic acid chain synthesized in step B) is in the same strand as the first template.
- 37. The method according to claim 36, wherein said synthesizing in step B) comprises:

B1) extending the 3' terminal of the first template by means of a polymerase having strand displacement activity to form the nucleic acid chain.

- 38. The method according to claim 37, wherein said extending in step B1) is carried out under conditions effective to permit the 3' terminal of the nucleic acid chain to include a sequence complementary to the 5' end portion of the first template, thereby forming a third region and a third complementary region that are substantially the same as the second complementary region and second region, respectively, and which, under suitable conditions, anneal to one another to form a third loop.
- 39. The method according to claim 38, wherein said extending in step D) displaces the sequence complementary to the 5' end portion from the 5' end portion, said synthesizing in step B) further comprises:

B2) further extending the 3' terminal of the nucleic acid chain, when the third region and the third complementary region anneal to one another to form the third loop, to include a sequence substantially the same as the single-stranded target region of the first template.

- 40. The method according to claim 39, wherein said synthesizing in step B) further comprises:
- B3) further extending the 3' terminal of the nucleic acid chain to include a sequence complementary to first template.





41. The method according to claim 39 further comprising:
annealing to the third loop a second oligonucleotide primer comprising
at the 3' terminal a nucleotide sequence complementary to the third loop; and
extending the 3' terminal of the second oligonucleotide primer by
means of a polymerase to form a second nucleic acid chain which includes a sequence
substantially the same as the single-stranded target region and a sequence complementary to
the single-stranded target region.

42. The method according to claim 29 wherein said synthesizing and said extending are carried out in the presence of a melting temperature regulator.

The method according to claim 42, wherein the melting temperature regulator is betaine.

The method according to claim 43, wherein 0.2 to 3.0 M betaine is allowed to be present in the reaction solution.

45. The method according to claim 29, wherein said providing in step A) comprises:

A1) annealing a first oligonucleotide primer to a sample single-stranded nucleic acid molecule comprising the single-stranded target region, the first oligonucleotide primer comprising a 3' portion which anneals to the sample single-stranded nucleic acid molecule and a 5' portion comprising substantially the same nucleotide sequence as an arbitrary region of the sample single-stranded nucleic acid molecule;

A2) extending the first oligonucleotide primer from its 3' end, using a suitable polymerase, to form a first single-stranded nucleic acid molecule comprising (i) a region complementary of the single-stranded target region, and (ii) a 5' end portion comprising the first region and the first complementary region which, under suitable conditions, anneal to one another to form a loop;

A3) displacing the first single-stranded nucleic acid molecule from the sample single-stranded nucleic acid molecule;

A4) annealing a second oligonucleotide primer to the first single-stranded nucleic acid molecule, the second oligonucleotide primer comprising a 3' portion which anneals to the first single-stranded nucleic acid molecule and a 5' portion comprising substantially the same nucleotide sequence as an arbitrary region of the first single-stranded nucleic acid molecule;

A5) extending the second oligonucleotide primer from its 3' end, using a suitable polymerase, to form the first template; and

A6) displacing the first template from the first single-stranded nucleic

acid molecule.

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46. The method according to claim 45, wherein the first oligonucleotide used during said first annealing in step A1) is the same as the oligonucleotide primer used during said annealing in step C).

47. A method of detecting a target nucleotide sequence in a sample comprising:

performing the method of amplifying according to claim 29 and determining whether the target region is present in the product of the method of amplifying.

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The method according to claim 47, wherein said determining

comprises:

combining the product of the method of amplifying with a probe containing a nucleotide sequence complementary to the first loop or the second loop; and observing whether hybridization occurs between the probe and the product of the method of amplifying.

The method according to claim 48, wherein the probe is labeled on particles and said observing comprises:

detecting whether an aggregation reaction occurs.

The method according to claim 47, wherein said performing the amplification method is conducted in the presence of a detector for nucleic acids, and wherein said determining comprises:

detecting a change in a signal of the detector.

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